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Survival and Growth of a Sheepshead Minnow Larvae and Juveniles on a Diet of 'Artemia' Nauplii

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Survival and Growth of Sheepshead Minnow Larvae and Juveniles on a Diet of *Artemia* Nauplii[1]

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ABSTRACT: Two geographic strains of brine shrimp (*Artemia franciscana*) nauplii were evaluated for nutritional quality as a food source for young sheepshead minnows (*Cyprinodon variegatus*). Although there was no mortality of fish fed either strain, fish reared on brine shrimp nauplii from San Pablo Bay, California, showed significantly greater growth than did those reared on nauplii from Macau, Brazil. The daily growth rate of sheepshead minnows measured over 32 days, was 0.42 mm/day.

Sheepshead minnows (*Cyprinodon variegatus*) 28 days old are used extensively by the U.S. Environmental Protection Agency in chronic and acute toxicity tests. Animals used for such tests should be physiologically similar from batch to batch to provide reproducible and comparable toxicity estimates. Nutritional history can alter the test animals' physiologic state and subsequent performance in a toxicity test (Mehrle et al. 1977).

Brine shrimp (*Artemia* spp.) are widely used as live food for rearing various marine animals. Previous studies have shown that different geographic strains of brine shrimp can have significantly different nutritional quality. Growth and survival of winter flounder (*Pseudopleuronectes americanus*) differed significantly when the larvae were reared on different strains of brine shrimp (Klein-MacPhee et al. in press), as did growth and survival of two species of crab larvae (*Rhithropanopeus harrisii* and *Cancer irroratus;* Johns et al. in press) and Atlantic silverside larvae (*Menidia menidia*; Beck et al. in press).

The purpose of the present study, conducted in early 1980 by the Environmental Protection Agency at Narragansett, Rhode Island, was to compare the nutritional quality of two strains of brine shrimp (*Artemia franciscana*) as measured by their effect on survival and growth of sheepshead minnows.

Methods and Materials

The two geographic strains of brine shrimp nauplii tested, one from Macau, Brazil, and the other from San Pablo Bay, California, were obtained from cysts supplied by the *Artemia* Reference Center, Ghent, Belgium. Nauplii were obtained by incubating the cysts in aerated natural seawater at 25°C and 30 ± 2.0 °/∞ salinity, filtered to remove 99% of all particles larger than 0.3 μm. Incubation took place in 4-L glass separatory funnels for 24 h. Newly hatched nauplii were separated from hatching debris and kept in aerated glass beakers until they were fed to the fish.

Sheepshead minnow larvae, used to compare the nutritional quality of the two strains of nauplii, were obtained from a spawning stock kept at the Environmental Research Laboratory, Narragansett. They were originally collected during fall 1979 by beach seine from Jerusalem salt marsh, Washington County, Rhode Island. Two males and five females were kept in recirculated water held in a 75-L glass aquarium, with undergravel biofilters providing continuous aeration and filtration. They were fed adult brine shrimp twice daily. Spawning took place over 5 days and the adults were removed before any larvae appeared. Mean hatching time was 8 days. Six-day post-hatch larvae (mean length, 8.14 mm; mean weight, 4.12 mg) were used in the experiment because that was the age at which the minnows began to swim freely in the water column. They were fed newly hatched Macau strain nauplii before use, because that had been found to be a superior food (Beck et al., Johns et al., and Klein-

[1]Contribution No. 182 from the Environmental Research Laboratory, Narragansett, R.I. This report, ISA-XXIII, is one of a series, International Study on Artemia.

MacPhee et al., in press).

There were three replicates each of fish fed Macau strain nauplii and fish fed San Pablo Bay strain nauplii. In addition, there was an unfed control, to test for the presence of any extraneous food source that might have been in the system.

The assay system was a set of cylindrical acrylic hatching jars, 45.7 cm high and 15.2 cm in diameter, holding 5.5 L of filtered (0.3 μm) seawater. The semi-static system was provided with mild aeration and one-third of the water volume was replaced daily. The hatching jars were placed in random order. The stocking density was 50 larvae per hatching jar. The number of mortalities and presence of any unconsumed food was observed daily. Temperature, salinity, and dissolved oxygen were monitored daily; light intensity was measured at the beginning and end of the experiment. Ranges of environmental variables were: temperature, 20.8 – 23.4°C; salinity, 30 – 33 °/$_{oo}$; dissolved oxygen 5.1 – 7.1 mg/L. Light was provided by fluorescent lights (470 – 560 lux) above the hatching jars set for a regime of 12L:12D.

Daily food ration for the larvae was 10% of the total wet weight of the fish in each jar on a dry weight basis. Mean wet weight at the beginning of the experiment was determined from 20 larvae sampled from the stocking group. An extra replicate of fish fed Macau nauplii was maintained for weekly determination of mean wet weight for recalculation of the diet ration. Mean wet weight was based on a sample of 15 fish from this replicate. After 18 days, the experiment was terminated and the total length and wet weight of each fish were recorded.

Sheepshead minnow larvae used to measure daily growth were obtained by the same spawning techniques described previously. After hatching, the minnow larvae were fed a diet of Macau strain nauplii twice a day. Ten fish were sacrificed daily and their total lengths, wet weights, and dry weights were recorded. Lengths were measured to the nearest 0.1 mm, using dial calipers, and weights were determined to the nearest 0.05 mg on an electronic balance. Dry weights were measured after drying 48 h at 60°C. Water temperature was monitored daily and ranged from 23.8 to 25.2°C. Salinity and dissolved oxygen were measured every 3 days and ranged from 30 to 32°/$_{oo}$ and from 5.7 to 6.5 mg/L, respectively. Light regime was 12L:12D and light intensity was 480 lux. After 32 days (which represents the growth period of fish reared 28 days, then used in a 96-h toxicity test) the experiment was terminated.

Results and Discussion

All larvae that were fed brine shrimp nauplii from both geographic sources were alive after 18 days. The lack of mortality in fish fed the San Pablo nauplii differs from the results found in previous comparisons of brine shrimp from Macau and San Pablo Bay. Johns et al. (in press) found that San Pablo Bay *Artemia* supported no survival of two species of crab larvae beyond the megalopa stage. Klein-MacPhee et al. (in press) found that survival of winter flounder larvae reared on San Pablo Bay *Artemia* was significantly lower than that of larvae reared on Macau *Artemia*. Beck et al. (in press) reported similar results for Atlantic silverside. Differences in survival of test animals fed different strains of brine shrimp may have been due to toxic contaminants in some strains (Olney et al. in press).

The high survival rate of sheepshead minnows on both diets in this experiment may have been due to the relative hardiness of the species. The sheepshead minnow apparently possesses a variety of physiological mechanisms that enable it to adapt to extreme environmental conditions. It tolerates temperatures as high as 39°C (Westman and Compton 1960), salinity as high as 142.2°/$_{oo}$ (Simpson and Gunter 1956), and the water in anaerobic springs with dissolved oxygen levels from 0.00 to 0.81 mg/L (Odum and Caldwell 1955). This species is apparently more able to withstand pollutants than most other species of shallow water fish that have been tested. Anderson et. al. (1974) found that sheepshead minnows were much more tolerant of oil than other fish in the bioassay and Eisler (1971) found that they had a 96-h TL50 for cadmium that is more than twice that of the striped killifish (*Fundulus majalis*).

It is interesting to note that 23 fish (46%) remained alive in the unfed control jar at the termination of the experiment, but they had not grown (mean length, 7.57 mm; mean weight, 3.73 mg). Survival of the unfed larvae may have been due to accidental introduction of brine shrimp cysts during the daily siphoning process. Also, a slight buildup of brown algae along the bottom of the hatching jar may have supported survival.

At the end of the experiment, fish fed San Pablo Bay strain nauplii had a mean weight ($\pm$ SE) of 84.18 $\pm$ 1.59 mg, which was significantly greater (Student's *t*-test; $P < 0.005$) than that for fish fed the Macau strain (77.39 $\pm$ 1.48 mg). Our findings are similar to those of Beck et al. (in press) for the Atlantic silverside. However, Klein-MacPhee et al. (in press) reported opposite results for winter flounder larvae. Differential growth of different species on diets of the two strains of nauplii suggests differences in their nutritional requirements, physiology, or both. Sheepshead minnows and Atlantic silversides reside primarily in very shallow salt-marsh habitats, whereas winter flounder larvae are characteristically found in open coastal waters. Different nutritional needs may simply be a reflection of adaptation to different habitats.

Schauer et al. (in press) showed that Macau strain brine shrimp have higher levels of long-chain fatty acids than do those from San Pablo Bay. A negative correlation thus is apparent between long-chain fatty acid levels and the growth of sheepshead minnows. Although marine fish generally require a diet high in long-chain fatty acids (Cowey and Sargent 1979), our results indicate that the San Pablo Bay brine shrimp nauplii are nevertheless a superior food for the sheepshead minnow.

Daily growth measurements of sheepshead minnows indicate that the total length of fish expressed as a function of time is a linear relation (Fig. 1).

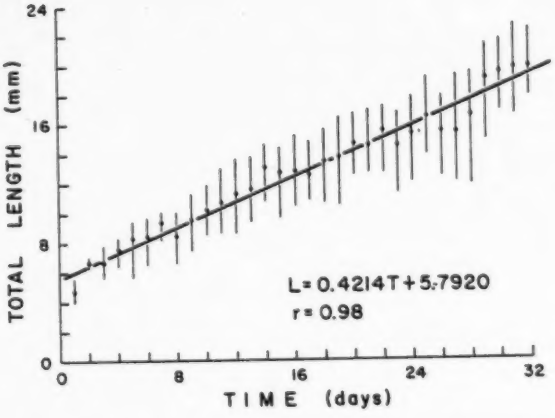

Fig. 1. Total length of young laboratory-reared sheepshead minnows as a function of time. Dots represent means of 10-fish samples, vertical bars represent ranges. L = total length; T = time.

Log wet weight as a function of log time (Fig. 2) and log dry weight as a function of log time (Fig. 3) are also linear relations. The following length-weight regressions were calculated from the experimental data:

$$\log (W) = 4.0518 \log (L) - 3.1034;\ r = 0.97$$
$$\log (D) = 3.6484 \log (L) - 3.2730;\ r = 0.98$$

where L = total length (mm), W = wet weight (mg), and D = dry weight (mg). Our results show that the average daily growth rate of sheepshead minnows in the laboratory (0.42 mm/day) was only slightly larger than that reported by Warlen (1964) for a natural population of fish. He calculated the growth rate of this species in the field to be 2.4 mm/week (0.34 mm/day) during the first month of growth. No statistical significance can be assigned to these differences because of the unknown variability of growth in the field.

The log-length/log-weight relations over the 32-day interval are remarkable in the size of the regression

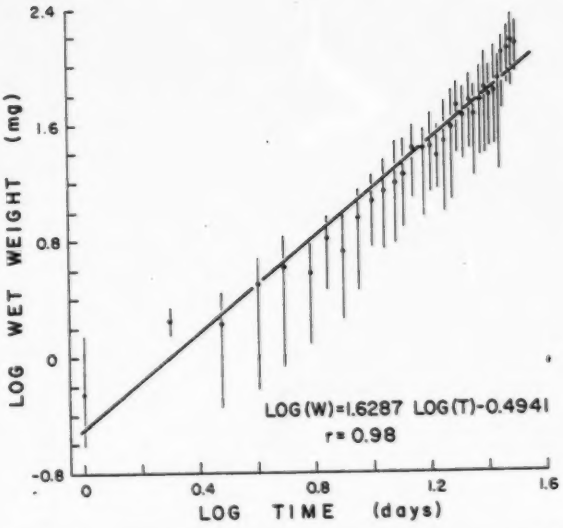

Fig. 2. Logarithm of wet weight of young laboratory-reared sheepshead minnows as a function of logarithm of time. Dots represent means of 10-fish samples, vertical bars represent ranges. W = wet weight; T = time.

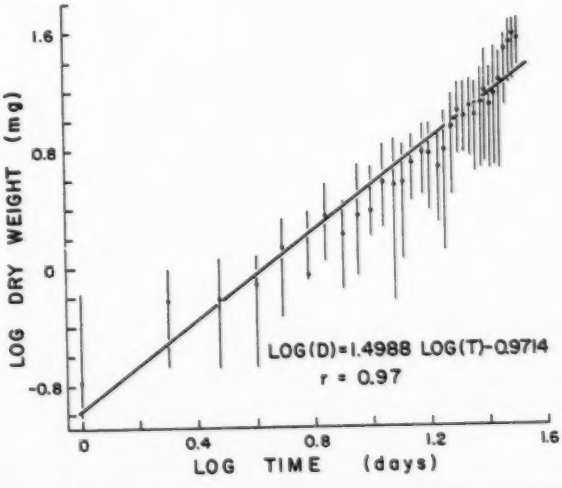

Fig. 3. Logarithm of dry weight of young laboratory-reared sheepshead minnows as a function of logarithm of time. Dots represent means of 10-fish samples, vertical bars represent ranges. D = dry weight; T = time.

coefficients involved. Typically, regression coefficients in log-length/log-weight equations approximate 3 (Ricker 1975). Our results may be due in part to rapid growth during the early portion of the life history and in part to the short, stocky body shape of the sheesphead minnow.

Growth of laboratory-reared fish was comparable to Warlen's (1964) measurements of growth in the field, so our estimates of length and weight changes with time can be used as a guideline in the 28-day laboratory rearing of sheepshead minnows for toxicity tests.

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